AMENDMENTS TO THE SPECIFICATION

Please insert the attached sequence listing (paper copy) in the specification after the drawings.

Please replace the paragraph beginning on page 2, line 2 and ending on page 3, line 1, with the following rewritten paragraph:

The invention will be described in closer detail in the following, with support of the enclosed examples and figures, in which

Fig. 1 shows the adherence values as a function of fibrinogen coating concentration for the *S. epidermidis* strains 2, 19 and JW27 (Example 1A),

Fig. 2 shows percent inhibition for antibodies against fibrinogen, compared to antibodies against fibronectin (Example 1B),

Fig. 3 shows percent inhibition as a function of competing fibrinogen concentration (Example 1C),

Fig. 4 shows the protease sensitivity of adherence to fibrinogen (Example 1D),

Fig. 5 shows the inhibition of adherence by LiCl extract (Example 1E),

Figures 6A-6E show ~~Fig. 6 shows~~ the complete nucleotide sequence of the *fig* gene from *S. epidermidis* strain HB and the deduced amino acid sequence of the encoded protein (SEQ ID NO:14). A putative ribosomal binding site (RBS) is underlined and a possible transcription termination hairpin loop is double underlined. A putative signal sequence (S) is indicated with an arrow and the translational stop codon with an asterix. The start of the non-repetitive N-terminal region called A, harbouring the fibrinogen binding activity is indicated by an arrow. R indicates the highly repetitive region. The 5 amino acid motif ~~LPXTG~~ involved

in cell wall anchoring is indicated in bold characters and the membrane-spanning region is marked M (Example 3),

Fig. 7 shows a schematic drawing comparing the fibrinogen binding protein FIG of *S. epidermis* and the clumping factor (ClfA) of *S. aureus*. The similarity, (%), of corresponding regions in the proteins is indicated in the figure between the two protein bars. S is the signal sequence; A, the non-repetitive region harbouring the fibrinogen binding activity; R, the diamino acid residue repeat region; W the region proposed to be involved in cell wall anchoring and M, the transmembrane domain. The numbers indicated refer to the amino acid positions in respective proteins as shown in ~~Figure 6~~ figures 6A-6E and 7 and in reference (McDevitt et al., 1994) (Example 3),

Fig. 8 shows how GST-FIG fusion protein is captured to fibrinogen in a dose dependent way (Example 10),

Fig. 9 shows the decrease of bacterial binding as a function of GST-FIG fusion protein, GST or FIG (Example 11),

Fig. 10 shows the relative adherence as function of serum dilution for two pre immune sera and a serum against GST-FIG and FIG, respectively (Example 12), and

Fig. 11 shows the relative bacterial adherence as a function of serum dilution for, on one hand, pre immune serum and, on the other hand, serum against GST-FIG (Example 12).

Please replace the paragraph beginning on page 7, line 8 and ending on page 7, line 21 with the following rewritten paragraph:

(A) Bacterial adherence

Fibrinogen was dissolved in PBS at 10 mg/ml and added in serial 3-fold dilution to microtiter wells (Nunc), from top to bottom. THe plates were incubated

overnight at room temperature (RT). To cover uncoated plastic sites the plates were coated with 2% bovine serum albumin for 1 hour at 37°C. The plates were washed with PBS with 0.05% ~~Tween 20~~ TWEEN 20 (PBST). Next, bacteria were added in serial 2- fold dilution in PBST, from left to right, to the fibrinogen coated microtiter plates. Bacterial adherence was allowed for 2 hours at 37°C or at 4°C overnight. Non-adherent bacteria were washed off and the bound bacteria were air-dried. The crosswise dilution of both fibrinogen and bacteria allows estimation of bacterial binding both as a function of fibrinogen concentration and of amount of bacteria. Determination of bacterial adherence was done by optical reading using a microtiter plate reader at A 405. The turbidity and light scatter caused by bound bacteria results in a reading ranging from 0.00 to 0.20. An example of adherence values as a function of fibrinogen coating concentration is shown in Figure 1 for three different strains (2, 19 and JW27). These conditions for adherence determination were used in the following experiments.

Please replace the paragraph beginning on page 9, line 24 and ending on page 10, line 5 with the following replacement paragraph:

Example 3: DNA sequencing and sequence analysis

Eight colonies coming from the second panning (pH 3.4) against fibrinogen described in Example 2 were chosen for further studies. Phagemid DNA from these colonies was prepared and partially sequenced. Seven of the clones seemed to contain the same insert. One of these seven clones called pSE100 was chosen for further studies. Purified phagemid DNA from the clone pSE100 was analysed by restriction mapping which revealed that the phagemid contained an insert of ~1.8 kilo base pair (kb). The nucleotide (nt) sequences of the complete inserts of pSE100 were determined and the nt and deduced amino acid (aa) sequences were analysed using the PC-gene program. This analysis

revealed that the insert of pSE100 contains an open reading frame of 1.745 nt (sequence list). Thus the insert encodes a ~~581~~ 582 aa protein, termed protein FIG (and the corresponding gene termed *fig*), with a calculated molecular mass of ~65 kDa (sequence list). Furthermore, the sequence analysis show that the insert of pSE100 is in the correct reading frame with the vector sequences in the 5'-and 3'-ends. This means that the insert gives rise to a fusion with the *pel* leader and the *myc* tail (sequence list) and that the native 5'- and 3'-ends of the *fig* gene is not present in the pSE100 clone.

Please replace the paragraph beginning on page 10, line 6 and ending on page 6, line 29 with the following replacement paragraph:

To obtain the missing 5' and 3' end of the fig gene a Southern blot analysis was performed using chromosomal DNA from strain HB digested with various restriction enzymes. The probe was prepared as follows; two oligonucleotides (5'CAACAACCATCTCACACAAC3' which is SEQ ID NO:1 and 5'CATCAAATTGATATTTCCCATC3' which is SEQ ID NO:2) were used to PCR amplify a ~1.3kb fragment from the insert of pSE100. The PCR generated fragments were 32P-labelled using random priming. After hybridisation using stringent conditions the NC-filter was washed and subjected to autoradiography. The result showed that the XbaI cleavage gave a single band in size of ~6 kb. The corresponding fragment was subsequently ligated into XbaI digested pUC18 vector. After transformation clones harbouring the ~6 kb XbaI-fragment were identified by colony hybridisation using the same probe as in the Southern blot experiment. One such clone, called pSE101 was chosen for further studies. DNA sequence analysis showed that the fig gene consist of an open reading frame of a 3291 nt, encoding a protein, called FIG of 1097 aa with a calculated molecular mass of ~119 kDa ~~(Figure 6)~~ (Figures 6A-6E). The FIG protein consist of several

typical features found among Gram-positive cell surface bound proteins, like a N-terminal signal sequence and a C-terminal 5 amino acid ~~aa~~ motif (indicated in bold characters at amino acid locations 1053-1057) ~~LPDTG~~, followed by a stretch of 17 hydrophobic aa ending in a stretch of charged aa (Figure 6). Following the signal sequence, there is a region, called A of 773 aa. The insert of pSE100 contains the sequence corresponding to residue 75 to 656 of the A region (Figure. 7). The A region is followed by a highly repetitive region of 216 aa composed of tandemly repeated aspartic acid and serine residues, called R ~~(Figure 6 and 7)~~ (Figures 6A-6E and 7). The dipeptid region consist of an 18 bp sequence unit (consensus of GAX TCX GAX TCX GAX AGX which is SEQ ID NO:3) repeated 36 times. The 18 bp sequence is almost maintained perfect throughout the whole R region except for the second unit which is truncated, consisting of only 12 of the 18 bp and the 3' end of the region where the consensus sequence is slightly disrupted (units 32, 34 and 36). The changes in the later units also result in an amino acid exchange which disrupt the DS repeat.

Please replace the paragraph beginning on page 13, line 12 and ending on page 14, line 5 with the following replacement paragraph:

Example 5: Western blot experiment

E. coli cells of strain TG1 and MC1061 containing pSEI00 were grown in LB (containing ampicillin and glucose) over night at 37°C. The next morning the cells were harvested by centrifugation, resuspended in LB (containing ampicilin, glucose and 0.1 M IPTG and further incubated at 37°C. Twelve hours later the cells were harvested by centrifugation and both the cells and the supernatant were taken care of. Four volumes of acetone were added to the supernatant and the resulting precipitate was collected by centrifugation, air-dried and resuspended in ice-cold PBS. Prior to electrophoresis the cells and the

precipitate from the supernatant were resuspended separately in a sample buffer containing 2.5% SDS and 5% beta-mercaptoethanol and boiled for two minutes. After denaturation the samples were analysed run under reducing conditions using the PHAST-system (Pharmacia) on a 8-25% gradient gel using SDS-buffer strips. After the electrophoresis was completed a NC-filter previously soaked in PBS was put on the gel and the temperature raised to 45°C. After ~45 minutes the NC-filter was wetted with 1 ml PBS, gently removed and placed in 15ml PBS containing 0.1% ~~Tween 20~~ TWEEN 20 solution (PBST 0.1%)for 30 minutes in RT (under gentle agitation and with two changes of PBST 0.1% solution). After the last change of PBST 0.1% fibrinogen was added to a final conc. of 20ng/ml and the filter was incubated for four hours at RT under gentle agitation. The filter was subsequently washed for 3x10 minutes using PBST0.1% and HRP-conjugated rabbit anti-human fibrinogen antibodies (DAKO code A 080, diluted 1:500 in PBST 0.1%) were added and the filter was incubated for 1 hour at RT under gentle agitation. After washing the filter 3x10 minutes using PBST 0.1% the bound fibrinogen was visualised by transferring the filter to a solution containing a substrate for the horse radish peroxidase (6 ml 4-chloro-1-naphtol (3 mg/ml in methanol) + 25 ml PBS + 20 µl H_2O_2). The result showed that a fibrinogen binding protein was found in both types of samples (cells and growth media) in both *E. coli* cells harbouring pSE100, while no such protein was found in the control cultures of *E. coli* TG1 and MC1061. The fibrinogen binding protein expressed from the pSE100 was in the approximate size as expected from the deduced amino acid.

Please replace the paragraph beginning on page 14, line 6 and ending on page 14, line 26 with the following replacement paragraph:

Example 6: The occurrence of the fig gene and the use of *fig* gene to identify *S. epidermidis* in diagnostic test

Purified chromosomal DNA from *S. aureus* strain 8325-4, *Streptococcus equi* subsp. *equi* strain 196 and subspecies *zooepidemicus* strain Z5, *Streptococcus pyrogenes* strain 2-1047, *Streptococcus dysgalactiae* strain 8215 were cleaved using the restriction enzyme *Eco*RI. The cleaved samples were run on an 0.8% agarose-gel together with chromosomal DNA from *S. epidermidis* strain HB cleaved with various restriction enzymes. After the electrophoresis was completed, the separated DNA fragments were transferred to a NC-filter using the Vacuum blotting system from Pharmacia. After the transfer the filter was hybridised under stringent conditions (in a solution containing 6xSSC, 5xDenhart, 0.5% SDS at 65°C) using a probe designed based on the nucleotide sequence of the insert of pSE100. This probe had earlier been prepared as follows, two oligonucleotides: (5'-AGGTCAAGGACAAGGTGAC-3' which is SEQ ID NO:4 and 5'CAACAACCATCTCAC ACAAC-3' which is SEQ ID NO:1) were ordered (Pharmacia) and used as a primer pair in a PCR (25 cycles of 94°C 1 minute, 50°C 30 seconds, 72°C 1 minute using an Perkin Elmer Cetus Thermal Cycler 480) to amplify an ~150 bp fragment of the insert of pSEI00. The amplified material was run on an agarose gel and the ~150 bp fragment was purified and radioactively labelled using ^{32}P-dATP and the Multiprime DNA labelling system (Amersham). The filter was hybridised over night and subsequently washed in a washing solution (0.2% SSC, 0.1% SDS) at 60°C and autoradiographed. The result showed that no hybridisation was detected in the samples originating from streptococci and *S. aureus* while hybridisation occurred to the samples coming from the *S. epidermidis* strain HB.

Please replace the paragraph beginning on page 15, line 1 and ending on page 15, line 16 with the following replacement paragraph:

Example 7: A PCR amplification assay for analysis of corresponding DS repeat regions from various isolates of *S. epidermidis*

McDevitt and Foster (Microbiology, 1995,141:937-943) have shown that the DS repeat region in various isolates of *S. aureus* strains may differ considerable. To investigate if the DS repeat region in *S. epidermidis* also varies in size between different isolates following experiment was performed. A pair of primers (5'CCGATGAAAATGGAAAGTATC3' which is SEQ ID NO:5 and 5' TCCGTTATCTATACTAAAGTC3' which is SEQ ID NO:6) hybridising on the 5' and 3' side, respectively, of the DS repeat region of protein FIG were used to PCR amplify the corresponding region in 11 different isolates of *S. epidermidis*. The amplification was performed as follows, after initial denaturation for 1 min. at 95°C a cycle started with a denaturation step for 30 sec. at 95°C, followed by an annealing time of 1 min at 50°C and a elongation period of 2 min. at 72°C. The cycle was repeated 25 times and ended in an final elongation period of 7 min. at 72°C. The PCR products representing the DS region of respective strain were analysed by agarose-gel electrophoresis. The result showed that one band of various length was present in each sample. The conclusion from this is that this type of method can be used as a diagnostic test to get a "fingerprint" of a particular strain. This might be useful in e. g. tracing a the origin of an infection.

Please replace the paragraph beginning on page 15, line 17 and ending on page 15, line 26 with the following replacement paragraph:

Example 8: The use of the DS fragment of strain HB to identify other homologous genes in coagulase-positive and -negative staphylococci

A DNA fragment consisting of the DS repeat region was constructed as follows. One pair of oligonucleotide primers (5'ACTGATCATGATGACTTTAGT 3' which is SEQ ID NO:7 and 5'TCCGTTATCTATACTAAAGTC3' which is SEQ ID NO:6) was used to PCR amplify the DS region of strain HB using the same conditions as described above. The amplification resulted in a ~700 bp fragment which was radioactively (^{32}P) labelled using random priming. This probe was used in a Southern blot analysis using chromosomal DNA (cleaved with EcoRI) from various species of *staphylococci* (*S. aureus, S. epidermidis* strain HB, *S. haemolyticus* strain 789 and strain SM131, *S. lugdunensis, S. schleiferi, S. intermedius, S. lentus, S. sciuri, S. carnosus, S. saprophyticus* and *S. hyicus*.

Please replace the paragraph beginning on page 16, line 1 and ending on page 16, line 17 with the following replacement paragraph:

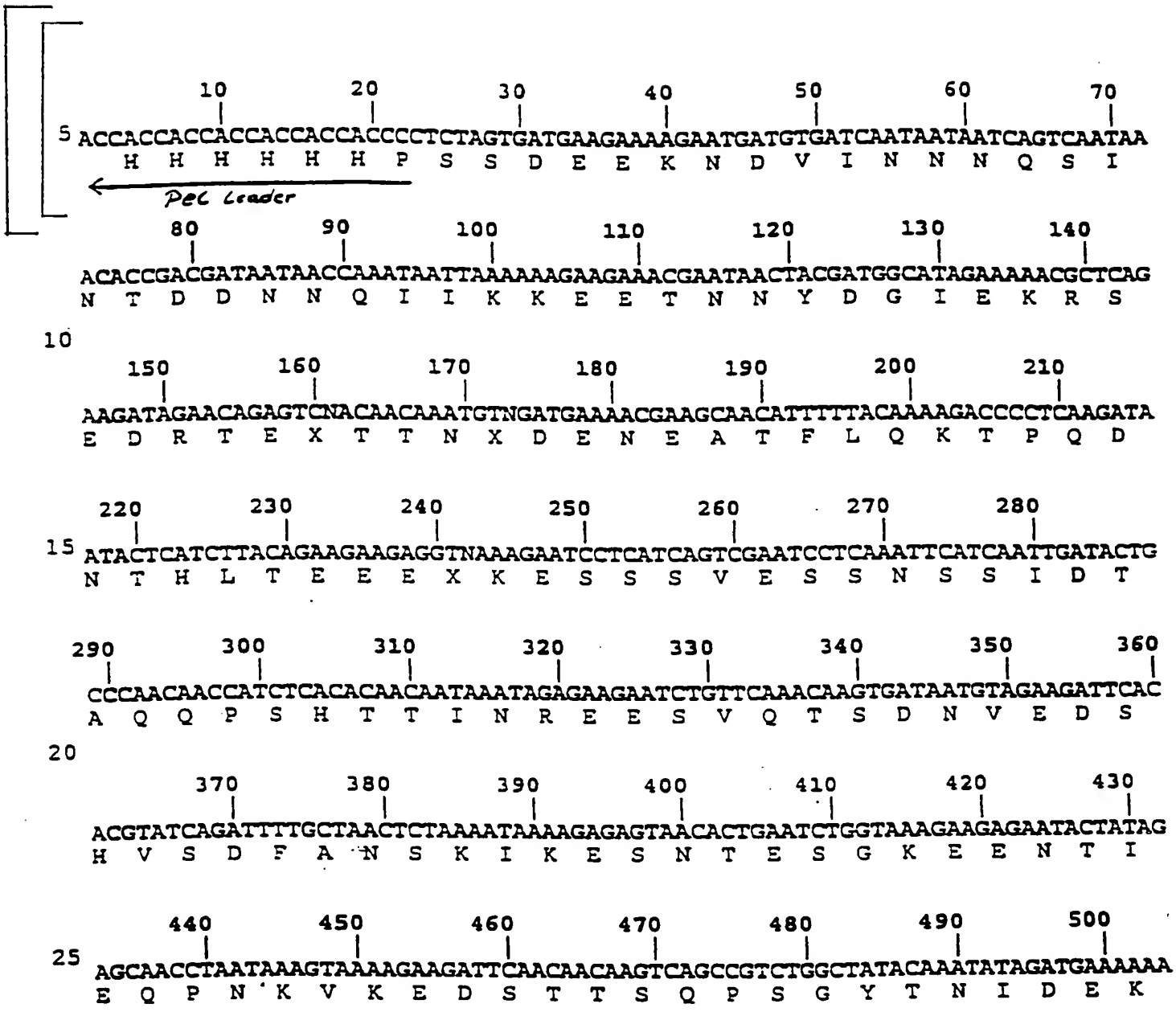
Example 9: Production of GST-FIG

By polymerise chain reaction, a DNA fragment was amplified encoding a portion of the fibrinogen binding protein. Upper primer was GCGGATCCAATCAGTCAATAAACACCGACGAT (SEQ ID NO:8) and lower primer was CGGAATTCTGTTCGGACTGATTTGGAAGTTCC (SEQ ID NO:9). Amplification was done for 30 cycles at 94°C 30 seconds, 60°C 30 seconds, 72°C 2 minutes beginning with 94°C for 4 minutes and ending with 72°C for 4 minutes. The amplified fragment was digested with EcoRI and Bam HI. Plasmid pGXT-4T (Pharmacia, Uppsala, Sweden) was digested with EcoRI and Bam HI, mixed with

the digested fragment and the mixture ligated using T4 DNA ligase according to standard procedures. The ligated DNA was transformed into *E. coli* strain TGI. A transformant was isolated with a plasmid encoding a fusion protein composed of glutathione thio transferase and fibrinogen binding protein. The protein was purified with the vector plasmid according to Pharmacia's instructions. The purified GST-FIG protein was subjected to Western affinity blot. It was run on polyacrylamide gel electrophoresis, transferred to nitrocellulose paper by passive diffusion, the paper treated with fibrinogen (5 µg/ml) for 2 hours at room temperature, followed by rabbit anti fibrinogen antibodies conjugated to HRP. A band corresponding to a molecular weight of approx. 100 kDa was seen. Omitting fibrinogen in a control experiment displayed no band.

Please replace the paragraph beginning with the term "Sequence List" on page 19, line 1 and ending with the last line of text on page 21, with the following replacement paragraph:

Sequence list (SEQ ID NO:10)



Sequence list cont.

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I  S  N  Q  D  E  L  L  N  L  P  I  N  E  Y  E  N  K  A  R  P  L  S  T

      580        590        600        610        620        630        640
      |          |          |          |          |          |          |
CATCTGCCCAACCATCGATTAAACGTGTAACCGTAAATCAATTAGCGGCGGAACAAGGTTCGAATGTTAACC
T  S  A  Q  P  S  I  K  R  V  T  V  N  Q  L  A  A  E  Q  G  S  N  V  N

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|          |          |          |          |          |          |          |
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           |          |          |          |          |          |          |
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D  A  E  N  L  I  Y  D  V  T  F  E  V  D  D  K  V  K  S  G  D  T  M  T

      800        810        820        830        840        850        860
      |          |          |          |          |          |          |
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V  D  I  D  K  N  T  V  P  S  D  L  T  D  S  F  T  I  P  K  I  K  D  N

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|          |          |          |          |          |          |
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           |          |          |          |          |          |          |
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      1160       1170       1180       1190       1200       1210       1220
      |          |          |          |          |          |          |
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Sequence list cont.

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                                                    Myc tail
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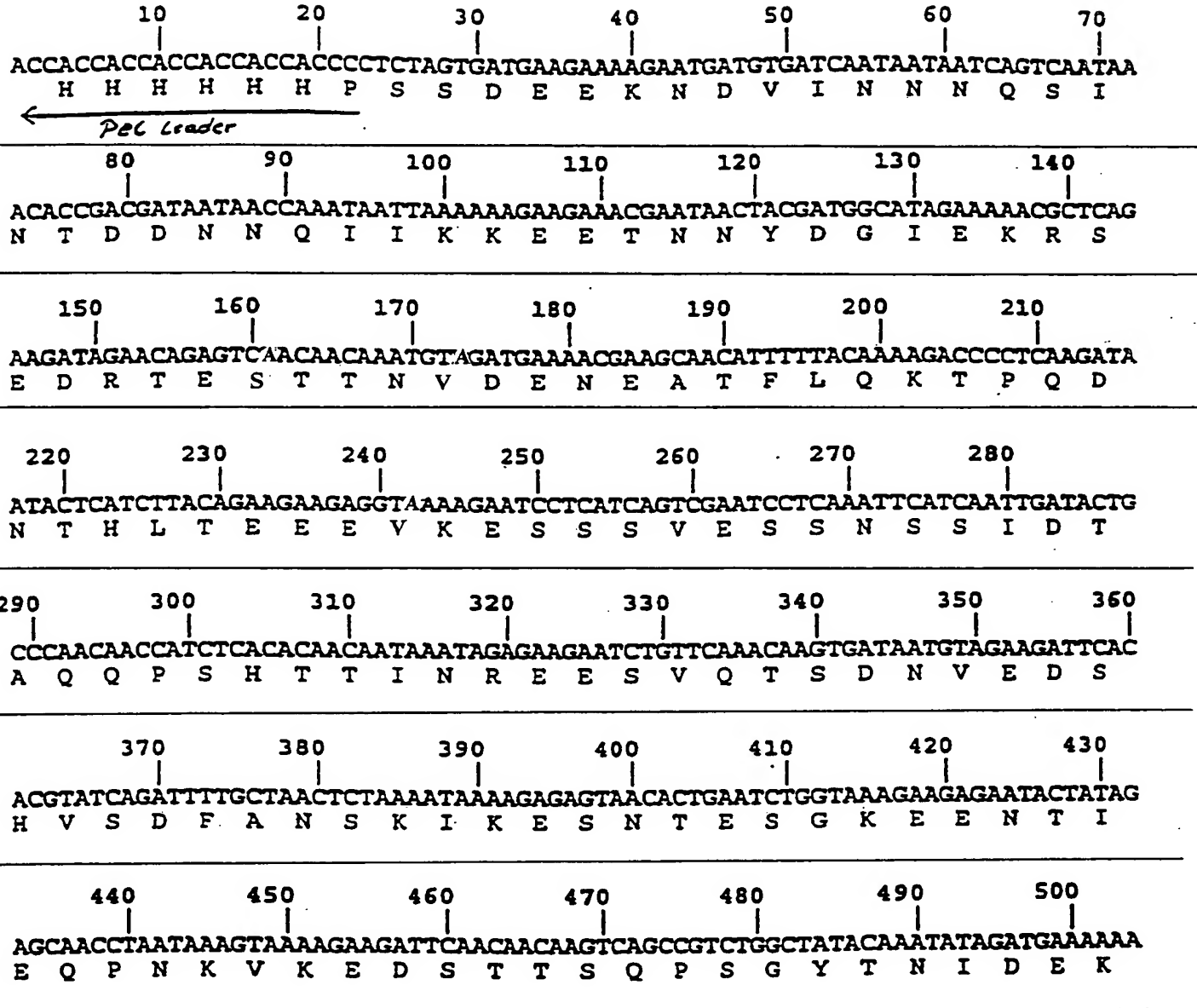

Sequence list cont.

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        580        590        600        610        620        630        640
CATCTGCCCAACCATCGATTAAACGTGTAACCGTAAATCAATTAGCGGCGGAACAAGGTTCGAATGTTAACC
T  S  A  Q  P  S  I  K  R  V  T  V  N  Q  L  A  A  E  Q  G  S  N  V  N

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        730        740        750        760        770        780        790
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800        810        820        830        840        850        860
TGGATATAGATAAGAATACAGTTCCATCAGATTTAACCGATAGCTTTACAATACCAAAAATAAAAGATAATT
V  D  I  D  K  N  T  V  P  S  D  L  T  D  S  F  T  I  P  K  I  K  D  N

870        880        890        900        910        920        930
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S  G  E  I  I  A  T  G  T  Y  D  N  K  N  K  Q  I  T  Y  T  F  T  D  Y

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Sequence list cont.

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1590        1600        1610        1620        1630        1640        1650
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D  L  P  P  E  K  T  Y  K  I  G  D  Y  V  W  E  D  V  D  K  D  G  I  Q

1660        1670        1680        1690        1700        1710        1720
ATACAAATGATAATGAAAAACCGCTTAGTAATGTATTGGTAACTTTGACGTATCCTGATGGAACTTCAAAAT
N  T  N  D  N  E  K  P  L  S  N  V  L  V  T  L  T  Y  P  D  G  T  S  K

1730        1740        1750        1760        1770        1780
CAGTCAGAACAGATGAAGATGGGAAATATCAATTTGATGGGGTGCAGGTCGAC
S  V  R  T  D  E  D  G  K  Y  Q  F  D  G  V  Q  V  D
                                                    Hyc tail →
```

Sequence list. A partial nucleotide sequence of the putative *fig* gene from *S. epidermidis* strain HB and the deduced amino acid sequence. The vector sequences in the junction of the 5'- and 3'-ends are indicated.

AMENDMENTS TO THE DRAWINGS

The attached sheets of drawings include changes to Figure 6. In particular, the attached sheets of drawings include Figures 6A-6B which replace the original sheets of Figure 6. The changes made to Figure 6 are explained below.

Originally submitted Figure 6 contains 5 pages with the first page labeled "Fig. 6" and the next four pages labeled "Fig. 6 continued". In the parent application no. 09/147,405 the examiner determined that Figure 6 is improperly labeled and required applicant to label the first page of the drawing as Figure 6A and the second, third, fourth and fifth continuing pages as Figures 6B, 6C, 6D, and 6E, respectively. The present amendment to Figure 6 makes these same changes in this application so that the drawings in this application are the same as the drawings in the aforementioned parent application.

Figure 6D also incorporates another change required by the examiner during the prosecution of the parent application. In particular, the examiner required that the letters "LPDTG" of Figure 6D should be shown by bold letters. Figure 6D was amended in the aforementioned parent application to comply with this requirement. Figure 6D submitted herewith also makes this same change.

The attached sheets of drawings also include corrected drawings for Figures 3, 7 and 11 wherein the corrections cure the defects noted by the draftsperson in form PTO-948 in the aforementioned parent application.

Attachment:

1. Replacement sheets for figures 6, 3, 7 and 11.

2. Annotated sheets showing changes to Figure 6.